

Time response of increases in muscle performance after Low-Level Laser Therapy (LLLT) in weight training practitioners

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Abstract

Many studies have demonstrated the effectiveness of low-level laser therapy (LLLT) on muscle performance. Nonetheless, ideal application parameters are not known yet. The interval from the laser application moment until the time of the exercise is amongst them. In addition, the literature does not offer adequate studies that analyse different interval times in the same test in humans. Thus, the present study proposes the examination of the two distinct intervals, from the irradiation moment to the instant of the exercise performance. Forty male volunteers were recruited and randomly divided into four groups - control, placebo, immediate laser and 10 min after laser – to participate in this study. The therapy was performed with laser at 830 nm, with a power of 30 mW, energy of 3 J, during a time of 100 seconds per point irradiation, in the belly of the biceps brachii muscle. The comparison of the groups consisted in the Max force analysis, muscular power and muscle fatigue tests executed in the isokinetic dynamometer, and the recruitment of fibers by electromyography surface (EMG) and blood lactate levels in the brachial biceps muscle of weight training practitioners. A significant level of 5% ($p < 0.05$) was considered for data analysis. The parameters adopted in the application of LLLT significantly increased muscle activity (RMS) in all protocols (peak force, power and muscular endurance). In addition, LLLT was also effective in maintaining muscle performance. It was concluded that the interval time from the moment of application of the LLLT until the performance of the exercise can influence in a different way the physical capacities tested.

KEYWORDS: Low-level laser therapy; Muscle performance; Muscle fatigue; LLLT.

Introduction

Weight training is one of the most practiced sports in the world¹. This type of training when performed correctly can result in a significant improvement in motor skills, cardiorespiratory functions and increase in muscle strength, causing an increase in general performance, enhancing physical valences such as strength, power and endurance².

There are many advances in Science to enhance the results and provide an adequate recovery. Among these advances, photobiomodulation (PBMT) has stood out for being a non-invasive and safe therapy.

Evidence has shown that photobiomodulation therapy (PBMT) before exercise can be used as a tool to increase performance and alleviate fatigue in humans³⁻⁵.

Photobiomodulation, from low-level laser therapy (LLLT), is considered a non-invasive therapy, and safe with LLLT, cellular energy can be altered by increasing the levels of ATP available in the tissue through oxidative phosphorylation. Mitochondria have photoreceptors (cytochrome c oxidase) that, upon receiving LLLT, promote increased ATP synthesis⁶. LLLT also promotes increased peripheral

microcirculation, favoring the use of aerobic energy and reducing blood lactate accumulation⁷⁻⁸.

Studies have demonstrated that these biochemical reactions can improve muscle functioning and delay the fatigue process⁸⁻¹⁶. However, today the big question that is asked about LLLT is no longer about its effectiveness in slowing down the FM process and increasing performance, but rather, what are the application parameters that can optimize these effects? Since this tool allows numerous configurations of clinical applications⁵. However, the application parameters are not yet known. Among these parameters is the interval from the application of the laser to the moment of the exercise. Currently in the literature there are few studies that have analyzed this application parameter, which makes it an unknown factor for

the action of LLLT on muscle performance⁹.

According to BARONI et al.⁸, ALMEIDA et al.¹⁵ and MACIEL et al.¹⁶, the interval of 0 to 3 minutes before the practice of the proposed exercise presents positive results on the muscular performance. On the other hand, ALBUQUERQUE-PONTES et al.⁹ presents in his studies in an animal model, that increased levels of ATP were observed 10 minutes after irradiation. Thus, higher levels of ATP can optimize muscle performance in physical tests. However, we believe that different interval times can have different effects on muscle performance (strength, power and endurance) and muscle fatigue.

Based on this premise, the present study aims to assess whether the interval time from laser application to the moment of exercise interferes with muscle performance.

Methods

This controlled, randomized, and double-blind study was realized at the Research and Development Institute (IP&D), at the Laser Therapy and Photobiology Center and at the Biodynamic Laboratory of the University of Vale do Paraiba (UNIVAP). The volunteers signed the declaration of free and informed consent and all their rights were protected. Forty out of eighty volunteers invited to the selection, were recruited for the study, according to the criteria established for the research. Forty volunteers (n = 40) were recruited as male weight training practitioners, aged between 20 and 40 years old. The protocol of this study was approved by the Research Ethics Committee of UNIVAP under number 07876/2015.

Sample Size

The sample size was calculated considering $\alpha = 0.05$ (5% type I error probable), $\beta = 0.95$ (95% of sample power) and data fatigue index reported in VIEIRA et al.¹⁴. The minimum number for each group was determined to be 5 volunteers, from which five participants were added to compensate possible dropouts during the study (amounting to 10 individuals per group)¹⁶ involving a total of 40 participants. This calculation used the principles of ARMITAGE and BERRY¹⁷, available on the online platform of the

Laboratory of Epidemiology and Statistics of the University of São Paulo (LEE - USP).

Inclusion/exclusion criteria

For one side the inclusion criteria were consisted by healthy Caucasian male volunteers, right-handed, weight training practitioners by a minimum period of 2 years, with a load of more than 4 hours weekly training, aged between 20 and 40 years old, and also have presented the BMI (Body mass index) $<25 \text{ kg} / \text{m}^2$.

On the other hand, subjects with neuromuscular history, osteomyoarticular, neurodegenerative or infectious injury, or those who underwent surgery within less than 24 months of recovery and also individuals with other comorbidities (heart failure, neoplastic diseases, restrictive diseases, among others) were excluded from the study.

Experimental design

The volunteers were randomly assigned to four groups which received the following treatment:

- Control (n=10): It was not realized any type of intervention before the tests were performed on the isokinetic dynamometer (ID).
- Placebo (n = 10): The laser treatment simulation was accomplished and then the tests

were performed in the ID.

- Immediate Laser (n = 10): They received the laser treatment and immediately after, without any interval, the tests in the DI were realized.

- 10 min after laser (n = 10): The volunteers received the laser treatment and after ten-minute interval, it was executed the tests in the DI.

The experiment was divided in 4 steps in a single day: the first stage consisted on a physical

evaluation and the first blood lactate test; in the second stage the placebo, immediate laser and ten-minute laser groups were irradiated; during the third stage, the tests were applied to all groups in the ID, when the signs of the brachial biceps muscle were collected through electromyography surface (EMG) simultaneously to the tests. In conclusion, five minutes later, the last blood lactate test was performed for all groups. All the stages considered above can be observed in FIGURE 1.

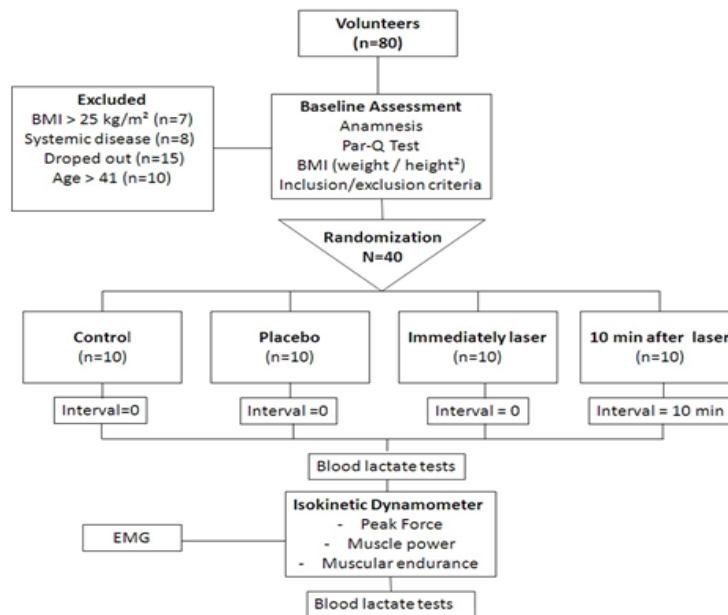


FIGURE 1 - Study flowchart.

Procedures

Firstly, the blood lactate was collected before and after five minutes the ID tests. For the collections it was used one monitor for determination of blood lactate (Accutrend®, Roche, Mannheim, Germany) and test strips (BM-lactate, Roche, Mannheim, Germany). After that, for each volunteer, the device was calibrated using the “master tape” (BM-lactate, Roche, Mannheim, Germany) and then the reading was performed, where each disposable experiment strip had its test zone covered by a blood sample. The blood sample was obtained from the volunteer through a disposable lancet (Softclix, Roche, Mannheim, Germany), adapted to a pen, in the distal phalanx middle finger region, which was pre-washed with neutral soap

and rinsed in running water before the analysis started. Next, it was made a local pressure with sterile gauze (Cremer, Santa Catarina, Brazil). Moreover, the blood lactate test was always fulfilled by the same evaluator.

With respect to the LLLT it was used a laser device (Twin Flex Evolution®, MMOptics, São Paulo, Brazil). Before irradiation, the biceps area was cleaned with alcohol 70% (Santa Cruz®, São Paulo, Brazil), in order to remove the existent oiliness on the skin. The phototherapy points were demarcated on the biceps brachii muscle with the use of a plastic template, containing perforations with 3 cm equidistant.

In total there were 6 application points in the belly of the biceps brachii muscle. Considering the procedure for the LLLT application, the volunteer was sitting with his right arm on a table and the other rested on his leg as in FIGURE 2.



FIGURE 2 - Scheme of LLLT application in the region of the biceps brachii muscle.

The laser tip was protected with PVC film (Easy Pack, Theoto, São Paulo, Brazil) and both, the applicator and the volunteer, used protective goggles (C,A 10377, Weld Steel, Rio de Janeiro, Brazil) as a biosafety measure. Both the parameters and the electromagnetic irradiation points were based on previous studies and on the specific literature^{8,11,15]} The parameters of the treatments that were used are described in TABLE 1.

Regards to the Max force tests and power and muscular endurance (muscle fatigue index) an ID was used (Biodex Medical Systems®, New York, USA). The volunteers positioning in the ID (FIGURE 1), the preparation and the equipment calibration followed the guidelines of the

standardization manual "Test and Rehabilitation System" provided by the equipment manufacturer. Afterwards, a warm-up was performed with ten repetitions of elbow flexion (concentric movement) at a speed of 210° s⁻¹¹⁸ so that the volunteers could familiarize with the equipment. The test itself, consist of 3 sets of elbow flexion on the ID, the first and second sets being composed of 5 repetitions. The first sets were completed at the speed of 60° s⁻¹ (for evaluation of Max force) and the second sets at the speed of 120° s⁻¹ (for muscle power evaluation, the last sets were 20 repetitions in the speed 240° s⁻¹ (to the evaluation of muscle fatigue index -MFI). It was used a 60 second interval between each sets^{18, 19}.

TABLE 1 - Laser parameters that were used.

Parameters	10 min after laser	Immediate Laser
λ (nm)	830	830
A (cm ²)	0,2	0,2
Ø (cm)	0,5	0,5
P (W)	0,03	0,03
t (s)	100	100
E (J)	3,0	3,0
DE (J/cm ²)	15,5	15,5
TI (min)	0	10
Diodo	GaAl As	GaAl As

Legend:
 λ - Wavelength;
 The area;
 Ø - Diameter;
 P - Power;
 t - Time;
 DE - Energy density;
 TI - Interval time.

On the evaluation of muscle activity, a two channels electromyography surface (EMG) (EMG 230 DL, EMG System of Brazil, São Paulo, Brazil) was used, with 2000 Hz sampling frequency and band-pass filter 20 Hz and 500 Hz, with the common rejection mode being greater than 120 dB. On the other hand, in the volunteers, disposable polyethylene bipolar electrodes double-type, with medical hypoallergenic adhesive, silver chloride, and pre-conductive hydrogel (Medi-Trace®, USA), Ag/AgCl bipolar contact (silver/silver chloride) were used. The distance between the electrodes was 20 mm (center to center), placed in the muscular belly of the biceps brachii according to the SENIAM protocol. Analyses of EMG data were done through software (Emg Works Analysis 3.1.5, Delsys®, Massachusetts, USA) and the RMS calculated from the average of the 3 sets performed¹⁴.

Randomization and blinding procedures

The randomization procedure consisted in a raffle of a card (A = Control, B = placebo, C = immediately laser and D = 10 min after laser) by the volunteers as well the presence of a researcher (PhD student), with more than seven-year-research experience, who did not participate in the data analysis. The volunteers were not informed of the cards meanings and this researcher, who

implemented the irradiation of all the volunteers, was instructed not to communicate the type of treatment. Furthermore, the volunteers (except the control group, who did not receive any type of intervention) used dark green protective glasses, sealed with an opaque card in front of the eyes. Thus, the treatment was concealed from the participants and from the researchers who analyzed the data.

Statistical analysis

Regarding the statistical analysis, the following data were used: A) maximum force, defined as the highest peak force value (expressed in N.m) together with the mean RMS value of the Max force test; B) Muscular power (expressed in Watts) in conjunction with mean RMS value of muscular power; C) muscle fatigue index (MFI), defined as drop in performance (expressed in%) in conjunction with RMS performance; D) blood lactate levels before and after the tests in the ID.

The data were analyzed using a software (GraphpadPrism V, GraphPad Software, California, USA), where the Shapiro-Wilk test was utilized to analyze the data distribution. The normality test revealed a non-parametric distribution. the Kruskal-Wallis test with Dunn's test (multiple comparisons) was used, considering a significance level of 5% ($p < 0.05$).

Results

As said before, the sample of this study was composed by 40 male-weight training-practitioner volunteers. During the experiment there was not any exclusion. The regular age was 26.02 ± 4.4 year old, the body mass index (BMI) $22.25 \pm 1.5 \text{ kg/m}^2$, the percentage of fat (% G) was $13.59 \pm 1.7\%$ and the mean value in the Maximum Repetition (MR) test in the biceps curl exercise was $26.5 \pm 1.3 \text{ kg}$.

The obtained data for Max force (peak force) were established in the first sets of five replicates in the ID. In the analysis, it was possible to observe that the immediate laser group presented the highest force peak mean, followed by groups 10 min after laser and placebo, compared to the control group. However, the intergroup analysis showed that there was a significant difference only in the peak force mean between the immediate laser and 10 min after laser groups ($p = 0.0327$), differently perceived from

the other groups which did not present statistically significant difference. (FIGURE 3).

The RMS analysis in the Max force test showed that the group 10 min after laser presented a statistically meaningful difference in relation to the control group ($p = 0.0008$) and to the placebo group ($p = 0.0012$) according to FIGURE 4.

The collected data for muscle power were established in the second sets of five repeats at the ID. In the inquiry, it was observed that the groups which were irradiated presented higher muscular power average when compared to the control and placebo groups, although any statistically expressive differences were revealed, as observed in FIGURE 3.

In the RMS consideration with regards to the muscle power test, it was observed an increase in RMS mean in the groups that were treated with laser, where the highest demonstration is referred

to the 10 min after laser group. According to FIGURE 3, the 10 min after laser group presented statistically significant differences in relation to the placebo group ($p = 0.0001$) and

the control group ($p = 0.0001$). The immediate laser group also revealed a substantial alteration in relation to the placebo ($p = 0.049$) and control groups ($p = 0.0210$).

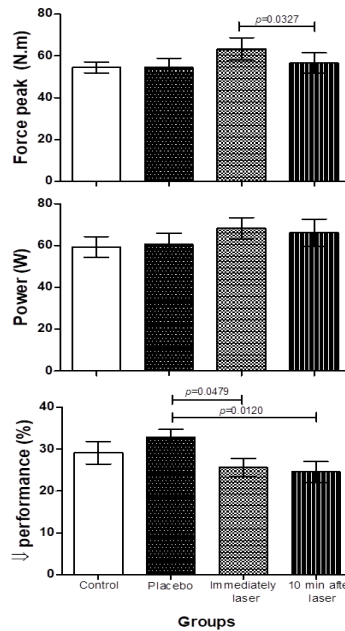


FIGURE 3 - Analysis of peak force, power and muscular endurance performed by ID.

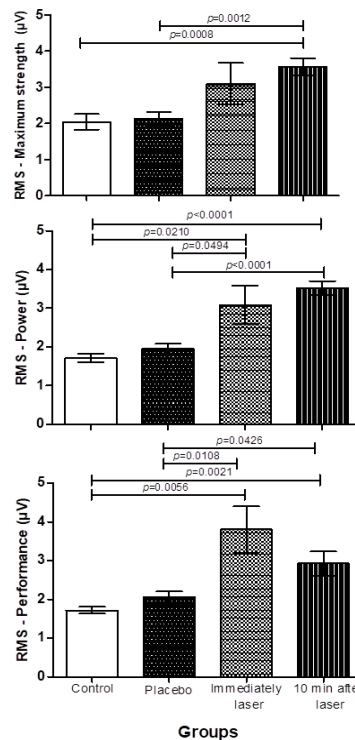


FIGURE 4 - Analysis of RMS in the of Max force, power and muscular endurance (performace) of the groups.

In the RMS consideration regarding to the MFI test, it was observed the increase of the RMS mean of the immediate laser group, followed by the 10 min after laser and placebo groups. The 10 min after laser groups showed statistically significant differences in relation to the placebo ($p = 0.0426$) and control groups ($p = 0.0021$). On the other hand, the immediate laser group also demonstrated a significant variance in relation to the control group ($p = 0.0056$) and the placebo group ($p = 0.0108$), as exhibited in FIGURE 4.

Lastly, the analysis of blood lactate, demonstrated the percentage increase (%) of the immediate laser and the placebo group basal lactate in relation to the control group. Accordingly, the immediate laser group presented, a statistically meaningful difference in relation to the control group ($p = 0.0208$). Yet, in the 10 min after laser group, blood lactate decreased when compared to the other groups, showing significant differences in relation to control ($p = 0.0161$), placebo ($p = 0.0255$) and immediately laser groups, as shown in FIGURE 5.

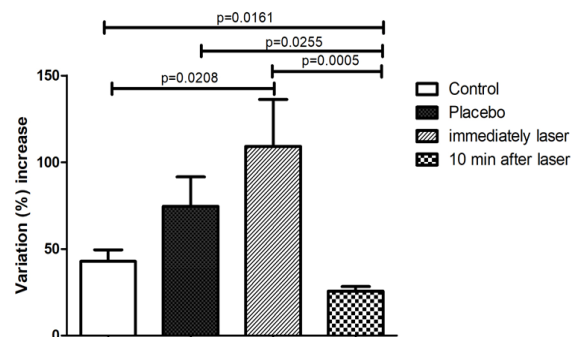


FIGURE 5 - Analysis (mean±standard error) of the variation in the increase in blood lactate levels.

Discussion

Several resources have been used in order to gain muscle performance. Among them stands the use of steroids and anabolic agents, which, consumed in an indiscriminate, stimulates the development of diseases and increase the risk of death of its users^{1, 20}. In this perspective, the studies that use LLLT have been widely disseminated due the positive effects on muscle performance. The LLLT is a non-invasive therapy that does not present adverse events if used appropriately²¹⁻²⁴. On the other side, many irradiation parameters have not been fully elucidated yet. Accordingly, due to de fact that there are not in the literature studies in humans that analyze different interval times in the same experiment, the present examination objective aims to identify the best interval from the moment of the LLLT to the proposed exercise, so that one could explore which of these intervals optimize muscle performance.

Therefore, the physiological variables evaluated in the present analysis were Max force, muscular power, muscle fatigue index, blood lactate levels and electrical activity of the biceps brachii muscle in weight training practitioners after the irradiation.

As a result, in the analysis of Max force and muscle power, it was found that the immediate laser group presented the highest mean in relation to the other groups, although any significant difference was confirmed in relation to the control and placebo groups. Corresponding to KELENCZ et al.¹² and KAKIHATA et al.²⁵, any substantial differences were represented regards to Max force and muscle power post-irradiation. Nevertheless, FERRARESI et al.²⁶, found a 28.76% increase in muscle performance in athletes submitted to MR test after LLLT. Accordingly, dos REIS et al.²⁷, reports that these divergences found in the literature happen due to the use of different LLLT parameters.

The present analysis employed energy of 3 J, which could justify of influence positively on maximum strength and muscular power. Studies that employed energy close to 1 J obtained positive results in the increase of the maximum strength and muscular power^{28,29}. Nonetheless, more studies addressing optimal irradiation parameters regards strength improvement and muscle power are

needed, considering that most research focuses on muscle endurance, that is to say, on the prevention and treatment of muscle fatigue. It has been also emphasized that the Max force, power and muscular endurance are distinct physiological variables, as in BOMPA et al.³⁰.

Moreover, in the present analysis, an expressive difference was observed in the examination of Max force in the immediate laser group in relation to the 10 min after laser group, although it was not found alteration amongst the treated and the control groups. In addition, in the MFI, analysis in which it is possible to evaluate muscular resistance and FM, the data are represented by percentage (%). Thus, the higher the percentage the higher the FM. Regarding this, a meaningful difference was observed in the irradiated groups, compared to the placebo group. Any difference was observed amidst the treated groups and the control group, though. It was also analyzed that the 10 min after laser group had the lowest percentage of where differences were not found in relation to the control group or placebo. FERRARESI et al.³¹, reported that an increased ATP in muscle tissue occurred over a range of 5 minutes to 24 hours.

The absorption process of electromagnetic radiation occurs inside the cells, where a photoreceptor called cytochrome c oxidase, found in the mitochondria, absorbs light, resulting in increased cellular metabolism^{32,33}. The LLLT may favor the increase of the peripheral microcirculation, which is obtained through the pre-capillary sphincter paralysis (infrared irradiation) or the degranulation of the mast cells (red irradiation)^{21,34}. The increased blood flow makes it possible to raise the supply of oxygen and other nutrients to the irradiated tissues, favoring muscle activity. In QUEIROZ et al.³⁵, performed in an animal model it was found an increase in the diameter of the vessels of the irradiated group from 20 to 40%, mainly in the first 5 minutes after the electromagnetic irradiation.

The electromyography surface is widely employed to evaluate muscle activity. The RMS can reflect the active motor units average number in a muscle in a particular interval^{6, 8, 24}. The electromyographic analysis results (RMS - tests of Max force, muscular power and resistance to muscular fatigue), indicate the increase in electrical activity and post-irradiation

muscle recruitment, where the irradiated groups presented significant differences contrasted to the placebo and control groups. In MUNÓZ et al.³⁶ and KELENCZ et al.¹², where the influence of LLLT on the masticatory muscles was evaluated, it was verified the increase in the electric activity of the muscles after the irradiation, corroborating with the present analysis. Nevertheless, other studies have also demonstrated the positive influence of LLLT in increasing muscular endurance, as found in LEAL JR. et al.³⁷, FERRARESI et al.³¹ and TOMA et al.³⁸. According to ALBUQUERQUE-PONTES et al.⁷, electromagnetic radiation may favor increased ATP synthesis, providing energy for metabolic processes, including muscle contraction and endurance.

Regards to the analysis of blood lactate the percentage (%) of the basal lactate level increase was evaluated. Accordingly, it was observed that the 10 min after laser group obtained a smaller improvement in relation to the other groups corroborating with the findings of BARONI et al.¹⁰, who analyzed the effect of infrared irradiation on the quadriceps muscle, using the fatigue protocol through the isokinetic dynamometer. The same results were found in REIS et al.²⁷, in which the action of LLLT on biochemical markers was investigated, with a small increase in blood lactate levels in the groups that received LLLT. Maciel et al.³⁹ emphasized that LLLTI in the near-infrared range, high-intensity physical pre-activity, may facilitate the removal of blood lactate and thereby reduce muscle damage, providing better athletes performance during their activities. In the immediate laser group, it was possible to observe the increase in blood lactate levels in relation to all groups. Based on what has already been discussed in the present study, it can be suggested that this increase in blood lactate levels may occur due to the probable increase in post-irradiation peripheral microcirculation, which is more pronounced in the first minutes, favoring muscle activity.

As proposed in this analysis the muscular performance has to be studied on different physiological parameters, such as Max force, muscular power and endurance, since there are biomechanical and biochemical differences between these physical valences.

Conclusion

As a final point, the present examination advocates that:

- In the analyzed parameters, LLLT did not promote significant difference on the muscular performance (Max force, muscular power and the muscular fatigue index);
- LLLT significantly increased muscle activity

(RMS) in relation to the other study groups (control, placebo, and immediately after LLLT), especially after the 10-minute therapy interval until the proposed exercise;

- There was a lower blood lactate rate, after the 10-minute LLLT interval until the proposed exercise, in relation to the other study groups (control, placebo and immediately after LLLT).

Compliance with ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. The authors declare that they have no conflict of interest.

Resumo

A intervenção da terapia a laser de baixa intensidade (TLBI) no tempo de resposta para o aumento do desempenho em praticantes de musculação.

Muitos estudos vêm demonstrando a efetividade da terapia a laser de baixa intensidade (TLBI) em relação ao rendimento muscular. Entretanto, os parâmetros ideais de irradiação ainda não são totalmente conhecidos. O objetivo do presente estudo foi analisar dois intervalos distintos, desde o momento de irradiação ao instante da execução do exercício. Participaram deste estudo 40 voluntários, com idade entre 20 e 40 anos do gênero masculino. Os voluntários foram divididos aleatoriamente em quatro grupos (controle, placebo, laser imediato e laser 10min). A TLBI foi realizada com laser em 830nm, com potência de 30mW, energia de 3J, durante um tempo de irradiação de 100 segundos, em toda a superfície do músculo bíceps braquial. A comparação dos grupos consistiu na análise de força máxima, potência muscular e fadiga muscular nos testes executados no dinamômetro isocinético, o recrutamento de fibras por meio da eletromiografia superfície (EMG) e níveis de lactato sanguíneo no músculo bíceps braquial de praticantes de musculação. Considerou-se um nível de significância de 5% ($p < 0,05$) para análise dos dados. Os parâmetros adotados na aplicação de TLBI aumentou significativamente a atividade muscular (RMS) em todos os testes (força de pico, potência e resistência muscular). Além disso, a TLBI também foi eficaz na manutenção do desempenho muscular. Com isso, concluímos que o intervalo de tempo desde o momento da aplicação da TLBI até a realização do exercício pode influenciar de forma diferente as capacidades físicas testadas.

PALAVRAS-CHAVE: Terapia com laser de baixa intensidade; Desempenho muscular; Fadiga muscular; TLBI.

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